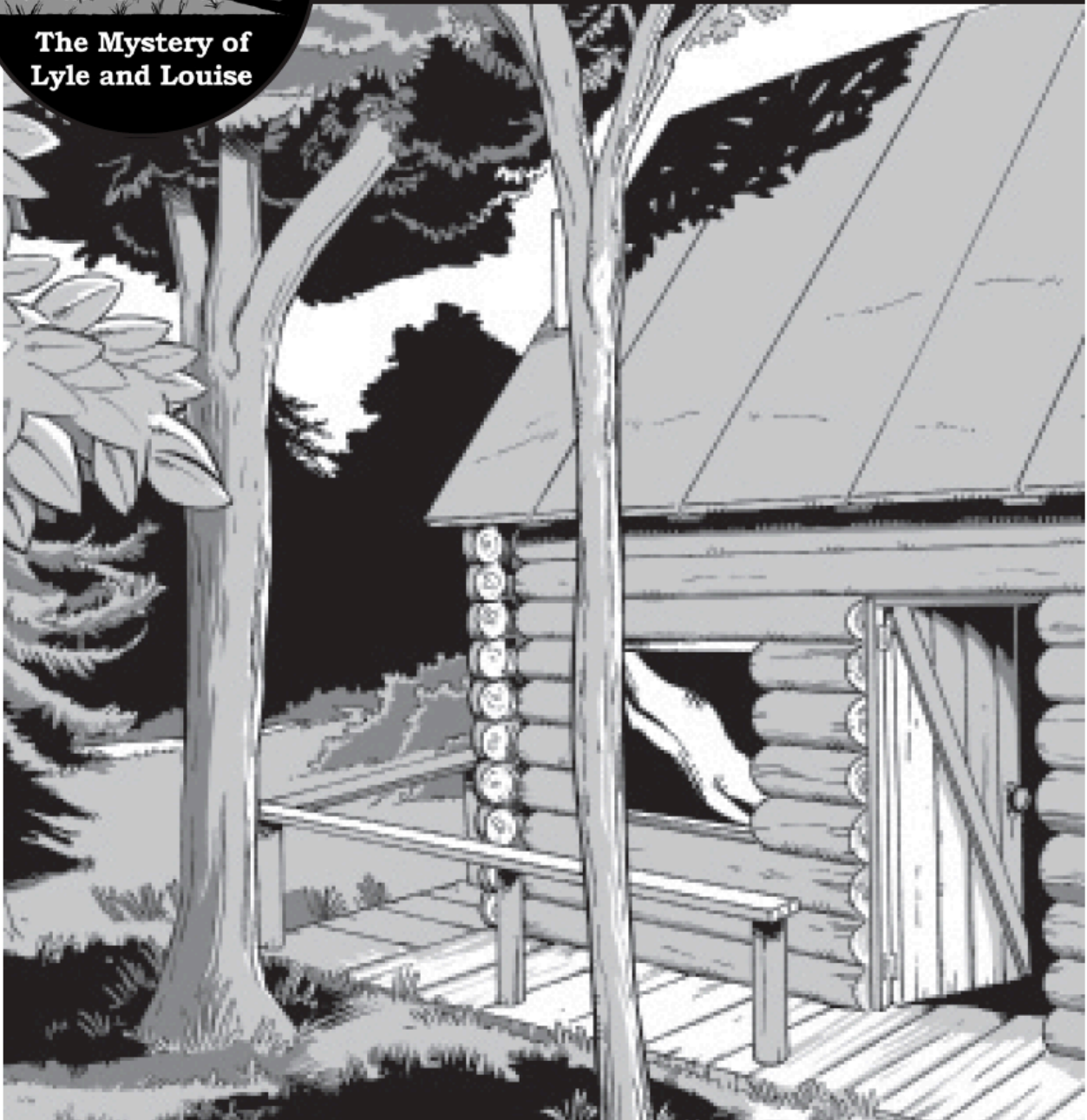




**The Mystery of
Lyle and Louise**

Stained with Suspicion

A Lab on Blood Detection & Evidence Processing



Rev. 7/1/2012

Blood Detection

BLOOD is a type of biological evidence frequently found at crime scenes that can be used to connect a suspect to a victim or object. Blood stains found at a crime scene can play a large role in eliminating or identifying a person as a potential suspect.

The two major components of blood are plasma and formed elements. 55% of the total blood volume is plasma, the fluid portion of blood, consisting of carbohydrates, lipids, hormones, inorganic salts, serum proteins (such as antibodies), and clotting elements. 45% of the total blood volume is formed elements, consisting of red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. The red blood cells, through the use of a protein called hemoglobin, are responsible for transporting oxygen to the tissues of the body, and, in turn, removing carbon dioxide from tissues. The white blood cells (0.1% of blood volume) play an important role in immune response and antibody production in the lymph nodes. Platelets (3.9% of blood volume) are responsible for initiating and participating in blood clotting.

The two main elements of blood used in forensic labs, with the exception of those performing DNA testing, are red blood cells and serum proteins. On the surface of the red blood cells are chemical structures called antigens that are grouped into systems determined by their relationship to one another. A commonly used antigen group system is the ABO group, which was used until the 1990s for blood typing. Serum proteins, such as antibodies, are frequently used for various tests. An antibody activates or destroys a specific antigen, which allows for particular reactions to occur when certain groups of antigens and antibodies are mixed.

In 1901, Karl Landsteiner discovered that blood could be distinguished by its group, or type, in what became known as the ABO group system. It was not until the early 1970's, however, that forensic scientists began to utilize these ABO blood groupings for clues that could help link blood to a specific individual. Blood type is considered class evidence be-

cause it is not unique to an individual the way that fingerprints are. The 4 blood antigen classifications are AB, A, B, and O. AB means that the blood has both A and B type antigens while O means that the blood has neither A nor B antigens. In addition to these antigens, the Rh factor helps to distinguish blood samples. This is either positive (Rh antigen present) or negative (Rh antigen not present) and appended after the A/B/O indicator, which means that there are 8 possible blood types (AB+, AB-, A+, A-, B+, B-, O+, O-).

Blood types vary greatly within the human population, but the rates of occurrence correlate strongly with race. Although blood typing can help link an individual to blood evidence, there are better ways to match an individual to a blood sample.

Type	African American	Asian	Caucasian	Hispanic
O+	47%	39%	37%	53%
O-	4%	1%	8%	4%
A+	24%	27%	33%	29%
A-	2%	0.5%	7%	2%
B+	18%	25%	9%	9%
AB+	4%	7%	3%	2%
AB-	0.3%	0.1%	1%	0.2%

In 1984 Alec Jefferys and his colleagues at Leicester University discovered that certain portions of the DNA structure of some genes (DNA markers) are as unique to an individual as fingerprints. Researchers have since developed different variations of the original Jefferys technique. These new techniques are called DNA profiling, or DNA typing. Forensic labs favor DNA analysis over ABO blood typing because it allows investigators to identify the person to whom the blood belongs. Blood detection tests are still performed in the field to identify a substance, but DNA analysis is the preferred confirmatory test once the substance has been identified as blood.

One of the most sensational cases involving DNA typing of blood evidence was the O.J. Simpson case.

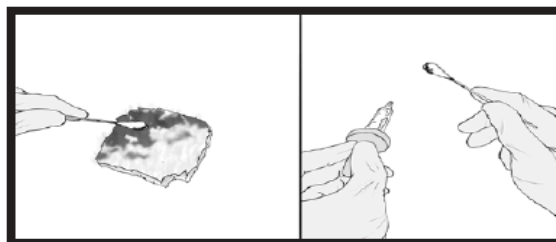
Blood Detection

On June 12, 1994, Nicole Brown Simpson and Ronald Goldman were murdered in an alley near O.J.'s home. A five drop blood trail was found near the bodies, and three additional blood stains were identified on the rear gate of O.J.'s Ford Bronco. These stains were collected, and analysis revealed that the blood belonged to O.J. Simpson. Results indicated a one out of fifty-seven billion statistical chance that this was not Simpson's blood. This level of precision is not possible with blood typing, but must be performed in a lab once blood has been detected.

When an investigator is confronted by a stain that looks like blood at a crime scene, it is difficult to know for certain that the stain is blood. After careful documentation, the investigator may quickly identify blood through a presumptive test at the scene. These tests are called presumptive because if a test result is negative, blood is absent, but if a test result is positive, blood is presumed to be present. As numerous compounds may cause false positive

reactions, a confirmatory test must be performed following a positive presumptive test. Confirmatory tests provide much more accurate results, but take longer to perform and require samples to be sent to a lab for analysis.

Presumptive tests are based on the peroxidase-like activity of hemoglobin contained in red blood cells. Peroxidases are enzymes that quicken the oxidation of a number of classes of organic compounds. There are two categories of presumptive tests: those that change color and those that cause a glowing reaction. In color change presumptive tests, a



There are many presumptive tests that can be used depending on the preference of the investigator, the forensic lab, and the situation. Some of the tests used are listed in the table below.

Presumptive Test	Indication of Positive	Situation Used	Reagents	False Positives
Phenolphthalein (Kastle-Meyer Test)	Bright pink color	On visible stains	Reduced phenolphthalein (phenolphthalin), hydrogen peroxide, in alkaline medium	Vegetable material (e.g. potatoes and horseradish)
Tetramethylbenzidine (TMB) / Hemastix	Green to blue-green color	On visible stains / Field tests	TMB, hydrogen peroxide, in acetic acid medium TMB, diisopropylbenzene dihydroperoxide, buffering material	Oxidizing agents, catalyst, and vegetable peroxidase Cosmetic substance
Luminol	Blue-white to yellowish green light	Latent blood	Luminol, sodium carbonate, sodium perborate	Plant enzymes, oxidizing agents, metals, and chlorine
Fluorescein	Fluoresce with alternate light source	Latent blood, vertical surface	Reduced fluorescein (fluorescein), hydrogen peroxide	Copper, hypochlorite

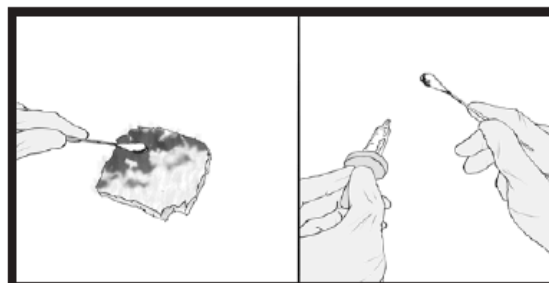
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sterile swab is moistened with distilled water and placed in contact with a small sample presumed to be blood. A drop of both a presumptive reagent and hydrogen peroxide is then added to the swab. An immediate color change indicates the possible presence of blood. Alternatively, a presumptive test may be performed by placing a thread or fragment of the dried material on a spot plate and adding the above reagents as in the swab test.

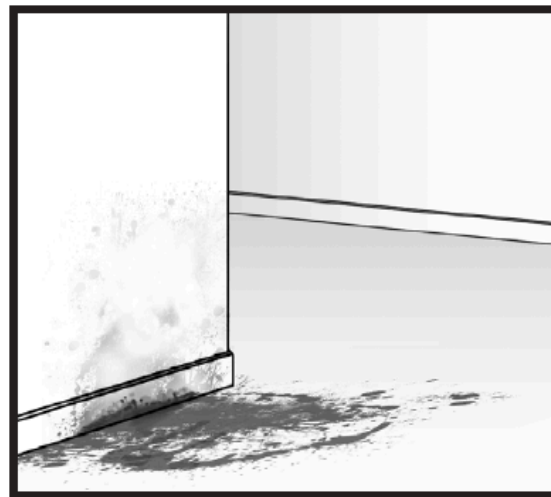
When performing the presumptive test, a substrate control test is required which will confirm that the test result is not brought about by the material that the stain was on. This is done by taking a swab of the original, unstained surface (as close as possible to the stain) and adding all similar reagents as the non-substrate control swab. Results for all presumptive tests must be recorded immediately before the sample is oxidized by air exposure, as this may result in a false-positive reading.

A phenolphthalein test, better known as the Kastle-Meyer test, is now one of the most frequently used presumptive color tests. In a positive reaction, reduced phenolphthalein will turn bright pink in an alkaline solution. This occurs because the phenolphthalein is oxidized by hydrogen peroxide in the presence of hemoglobin. Phenolphthalein reagents, however, have been known to give false positives when vegetable materials are present. As a result, after the evidence is collected and transported to the lab, a confirmatory test is performed.

The Hemastix test is another commonly used presumptive test for blood. This test is performed with commercial Hemastix strips, plastic strips with a reagent-treated filter paper at one end. Tetramethylbenzidine (TMB), the reactive reagent on Hemastix strips, is another well-known presumptive reagent. This product designed as a urine dipstick test, has been adapted to be used in the field for the detection of blood. To perform this test a swab is moistened with distilled water and placed in contact with the stain. The swab is then placed onto the tip of the dipstick; if blood is present, the Hemastix strip will turn green.

In some cases bloodstains can not be seen under normal lighting and viewing, such as a stain that has been cleaned up by the suspect. Therefore, before performing any tests, a high intensity light source is used to detect latent bloodstains. If a stain cannot be visualized with a light source, Luminol or Fluorescein tests may be used.

Luminol is highly sensitive, and is known to detect blood that has been diluted up to 1 in 10,000,000 times, unless a solvent such as bleach was used. It works similar to other color tests in that Luminol and an oxidizer (hydrogen peroxide) are applied to the bloodstain. This results in the oxidation and chemiluminescence of Luminol, producing a blue-white to yellowish green light when the treated area is darkened. Although the Luminol reagent is known to negatively impact some serologic testing processes, it does not affect most subsequent blood typing or DNA analysis. Still, the dilution of blood through the use of Luminol can make some genetic analyses difficult, and Luminol has been known to produce false positive results with plant enzymes, oxidizing agents, metals, and chlorine.



Fluorescein has been used to detect blood since as early as 1910. Reduced Fluorescein (fluorescein) is applied to a suspected stain and will fluoresce when an alternate light source is used. Unlike Luminol, Fluorescein is capable of revealing bloodstains that have been cleaned using solvents such as bleach.

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Additionally, Fluorescein is thicker than Luminol and, therefore, can be applied to vertical surfaces.

It is crucial that bloodstains found at a crime scene are documented, collected, tested, preserved, and analyzed correctly, as failure to perform each task properly can weaken or destroy potential evidence. The testing procedure is designed to reveal if the stain is blood, whether it came from an animal or human, and, if it is of human origin, how closely the blood can be linked to an individual.

The results of the presumptive test can assist the investigator in collecting the bloodstains. If the test was negative, only two or three samples from the stain must be collected. Investigators collect the stain sample by, preferably, transferring the whole item, or extracting the blood using one of several methods. The most common method involves taking a sterile, moistened swab or thread and rolling/swabbing the bloodstain. The swab or thread is then completely dried and placed in a paper bag, envelope, or box. Another well-known method is tape lifting the bloodstain. Fingerprint tape can be taken and used to carefully lift the bloodstain, which is then placed on vinyl acetate backing.

Proper evidence packaging is crucial to protect against loss, contamination, deterioration, cross-transfer between the samples (suspect/scene/item/victim), and biohazards.

All biological materials must be completely dried and placed in their own separate, correctly labeled, paper bags. Plastic bags are only used for transporting moist blood evidence for no more than two hours. If moist biological evidence is left in any plastic container there is a great possibility of microorganism growth which may alter the evidence, degrade DNA, and/or inhibit future testing. Collected bloodstains should be refrigerated, unless the bloodstain was found in soil, then it should be frozen so that microorganisms present will not degrade the DNA.

The evidence collection bags must be labeled properly with a description of the evidence, the source

location, agency, chain of custody, case / item numbers, health hazards, and storage conditions (room temperature, frozen, refrigerated). The protocols for evidence collection are very detailed and beyond the scope of this manual, however, it is important to remember that evidence which is improperly collected may be suppressed in court, which may cause the case to be dismissed.

After the evidence is collected and transported to the lab, a confirmatory test must be performed. Confirmatory tests are often microcrystalline tests that are based on the formation of hemoglobin-derived crystals under heated conditions. Microcrystalline tests involve the addition of specific chemicals to blood so that crystals with hemoglobin derivatives will be formed. This crystal formation is then observed microscopically. The two most common confirmatory tests are the Takayama and the Trichmann tests.

The Takayama test is performed by the addition of an alkaline solution with a specific structure of hemoglobin to the stain on a microscope slide. If blood is present, pink crystals will be observed as the slide is heated. The Trichmann test is performed by adding a small amount of chloride-containing glacial acetic acid to the stain on a microscope slide, and, if blood is present, small crystals are observed as the slide is heated. As with presumptive tests, the analysis of controls, specifically a positive control, is required for comparison.

Once a stain has been confirmed as blood, the forensic serologist must determine whether it is of human or animal origin. The standard test used in this determination is the precipitin test. The precipitin test is characterized by the formation of a precipitate due to an antibody that reacts with its corresponding antigen. Human antiserum, containing antibodies that specifically react with human antigens, reacts with human blood to form a precipitate.

Following the identification and characterization of the bloodstain, it must be analyzed to associate it to a particular individual. Blood factors, such as

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the ABO group, or DNA typing can assist in linking a bloodstain found at a scene to an individual.

Though forensic scientists currently have various tests that can be used to detect and analyze blood, advancements are continually being made. Blood is a complex system and scientists are constantly discovering new information and techniques to handle this evidence.

Glossary

ABO Group System: A classification system which has been widely used since the early 1970s. The ABO group system uses blood type to link blood to an individual. This system has not been widely used since the 1990's.

Confirmatory Tests: These tests are often microcrystalline tests that are based on the formation of hemoglobin-derived crystals under heated conditions. A confirmatory test would prove the presence of blood, but it is much more costly and time-consuming than a presumptive test.

DNA Typing: A classification system that has been predominantly used since the 1990's. It is based on research that proves that certain portions of the DNA structure are as unique to an individual as fingerprints. This system can accurately link one person to a blood sample.

Formed Elements: Formed elements make up forty-five percent of the total blood volume. The formed elements are red blood cells, white blood cells, and platelets.

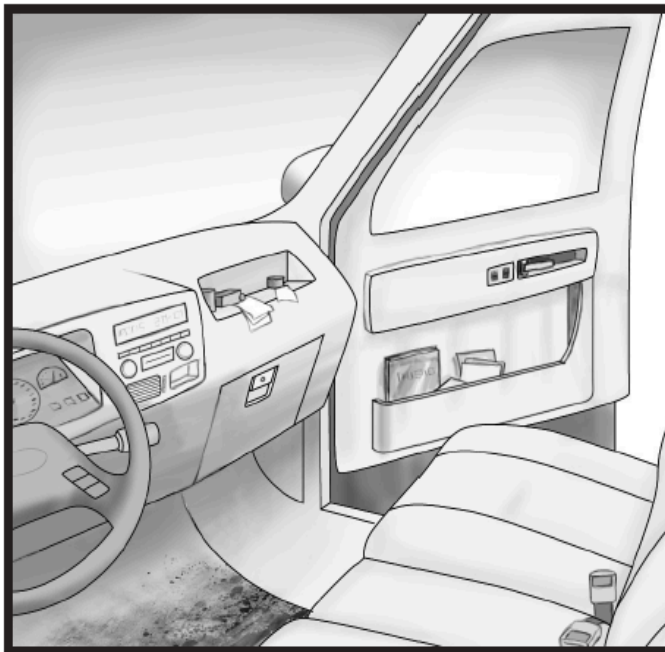
Plasma: Plasma is the fluid portion of blood which makes up fifty-five percent of the total blood volume. Plasma consists of carbohydrates, lipids, hormones, inorganic salt, serum proteins (such as antibodies), and clotting elements.

Red Blood Cells: Red blood cells are responsible for transporting oxygen to the tissues of the body and remove carbon dioxide from the tissues.

Presumptive Tests: These tests can confirm the absence of blood with a negative result. A positive result indicates the probable presence of blood, but, due to false positives, a positive presumptive test result must be confirmed with a confirmatory test.

The Evidence

An abandoned, blue Ford Ranger bearing the Tumbling Water Land Development Co. logo was found in New Mexico with its gas tank completely empty. As the New Mexico authorities examined the truck for potential evidence, they found suspicious smudges on the driver's side floor. At first glance, the smudges appeared to be mud, but upon closer examination, one investigator noted that he could see traces of a reddish substance mixed in with the mud. Therefore, thinking the stain could possibly be blood, he photographed the evidence and removed the suspected area of carpet to allow it to be examined at the lab.



Persons of Interest

The Mondelos

Louise Ann Mondelo, the 38 year old wife of Lyle Mondelo and mother of Wally and Jan, is also one of the owners of Tumbling Water Land Development Company. Friends say that Louise was in an unhappy marriage and had recently filed for divorce.

Lyle Christopher Mondelo, the 40 year old husband of Louise Mondelo and father of Wally and Jan, is a part owner of Tumbling Water Land Development Company along with his wife.



John Wayne Gretzky

John Wayne Gretzky is 41 years old. He is a friend and business partner of the Mondelo's in the Tumbling Water Land Development Company. According to rumors, John Wayne and Louise had a brief affair when Lyle and Louise first moved to Highland Park. He is known around town to be a greedy businessman, and has been suspected of shady deals in the past.



An unknown woman of similar height and build has been identified as Louise Mondelo. Although her identity is uncertain, this other woman was found either driving the Mondelo family car with two children preliminarily identified as Wally and Jan, or in a remote fishing cabin with a man who has been preliminarily identified as Louise's husband Lyle Mondelo.



Pre-Lab Questions

1. What are the two major components to blood?

2. What test did forensic scientists use to link blood to an individual until the 1990's

3. What test is now used extensively to link blood to an individual?

4. Why are blood detection tests still important in current investigations?

5. How do investigators find bloodstains that are not immediately visible under normal lighting?

6. Why are presumptive tests performed before confirmatory tests?

7. If a presumptive test has a positive result, can investigators guarantee that blood is present?

Lab Procedure

Lab 1: Presumptive Testing for Blood

1. Prior to performing presumptive tests on the evidence from the pickup truck, you will practice the presumptive tests using some positive controls, as well as some substances which also can give a positive result. Wear gloves when handling these chemicals.
2. Cut each card in half in the middle of the stain. This will allow you to repeat the experiment in case there is any confusion about the results.
3. Place the card half on top of a blank sheet of paper so that the reaction may be easily noted.
4. Add one drop of distilled water to the cotton swab and rub it into the stain upon the control card.
5. Add one drop of phenolphthalein solution to the cotton swab. If any color change occurs at this point then the reagent is contaminated and the test should be considered invalid.
6. Add one drop of the hydrogen peroxide solution to the cotton swab.
7. A pink color should appear between 30 seconds and three minutes to indicate that the dried material is most likely blood.
8. If a pink color is not observed or appears after three and a half minutes have passed, the test is considered negative.
9. Record your results on your Data Collection Sheet.
10. Test all substances on the provided cards in the manner stated above, recording predictions and reactions of each one.
11. Test other teacher-provided substances in the same manner, adding the water, phenolphthalein, and hydrogen peroxide. Observe and record the reactions. HINT: If additional substances are to be tested, tests may be performed upon both ends of the provided cotton swabs. Compare your shade of pink to the swab from the positive control card if you are unsure if you are getting the right color to indicate a positive result.

Lab 2: Processing the Evidence

1. Obtain your evidence from your teacher, signing and dating in the appropriate location on the Chain of Custody portion of the Evidence label.
2. Carefully cut open your evidence, opening it at an end that is NOT sealed by evidence tape.
3. Examine your evidence. Measure the stain and record several detailed observations about your evidence, including size, shape, color, and any other pertinent details.
4. If available, use a digital camera to take three or four pictures of the evidence from different angles.
5. If available, use a magnifying glass to look closely at the carpet square for other materials on the fabric.
6. Sketch the evidence noting at least two areas of the stain to be tested upon your sketch.
7. Take a cotton swab and wet it with distilled water. Rub the cotton swab onto the first area of the stain to be tested.
8. Perform the presumptive test on the cotton swab. Add one drop of phenolphthalein and observe the reaction. Add one drop of the hydrogen peroxide solution. A pink color should appear between 30 seconds and one minute to indicate that the dried material is most likely blood. If a pink color is not observed or appears after three minutes have passed, the test is considered negative.
9. If possible, take a picture of the color change observed.
10. Repeat the test on the sample from another part of the stain.
11. Determine whether the substance on your evidence is blood.
12. Complete your Data Collection sheet.
13. When you have reached a conclusion, return your stain to the evidence wrapper and reseal it.

Data Collection and Calculations

Lab 1:

Substance	Prediction: Positive or Negative?	Lab Observations (color of development, time to see pink)	Lab Result: Positive or Negative?
Positive Control			
Negative Control			
Substance #1			
Substance #2			

Lab 2: Record 4 or 5 physical details about evidence:

Draw a sketch of your evidence. Include measurements.

Describe your procedure for processing the evidence and the results you see.

Post-Lab Questions

1. What did you observe when you tested the positive control card?

2. What did you observe when you tested the negative control card?

3. What common food items provided a false positive in the presumptive blood test?

4. Why do police officers perform a presumptive test in the field? Based on your experiments, why is it important to do a confirmatory test later?

5. What did you learn about correctly processing evidence? Why is this procedure important?

6. What did your group conclude about the stain on the carpet? Did your test detect the presence of blood?

7. Based on your knowledge of the crime(s), what is your hypothesis about the events surrounding the substance on the carpet of the truck?
